Anatomy and Histology of Reproductive Organs of Female *Homalodisca coagulata* (Hemiptera: Cicadellidae: Proconiini), with Special Emphasis on Categorization of Vitellogenic Oocytes

NATALIE A. HUMMEL,[1] FRANK G. ZALOM,[2] AND CHRISTINE Y.S. PENG[2]

Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616

Ann. Entomol. Soc. Am. 99(5): 920–932 (2006)

ABSTRACT The anatomy and histology of female *Homalodisca coagulata* (Say) (Hemiptera: Cicadellida: Proconiini) reproductive organs is described using light microscopy. The reproductive organs of *H. coagulata* consist of one pair of ovaries, each with 10 telotrophic ovarioles, a pair of lateral oviducts, a common oviduct, a spermatheca, an enlarged genital duct, a complex bursa copulatrix, a vagina, two types of accessory glands, and a genital chamber. The reproductive organs follow the general pattern seen in cicadellids. The complex bursa copulatrix, important in copulation and sperm transfer, is described. A set of morphological criteria were selected, based on the stage of oocyte development, and used to evaluate and assign the rank of ovarian development for field-collected individuals and to assess the overall reproductive status of female insect populations. A principal component analysis of morphological and physiological characteristics suggests that the ovarian ranks reflect the reproductive status of the females. Understanding reproductive status and patterns is critical for determining the optimal time to implement control methods to suppress *H. coagulata* populations in southern California.

KEY WORDS glassy-winged sharpshooter, leafhopper, oogenesis, sperm, telotrophic

The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae: Proconiini), is a serious pest of many tree and vine crops and is known to vector the bacterium *Xylella fastidiosa* Wells et al., 1987 (Turner 1949, Wells et al. 1987), which causes disease in many crops, including grapes (Hewitt et al. 1946, Alderz and Hopkins 1979), almonds, and oleanders (Davis et al. 1980, Costa et al. 2000). Adult *H. coagulata* can vector *X. fastidiosa* throughout their life, whereas nymphs lose the ability to vector *X. fastidiosa* after each molt, but they can reacquire *X. fastidiosa* from infected hosts (Almeida and Purcell 2003).

H. coagulata was first detected in southern California in 1989 (Sorenson and Gill 1996), rapidly infested 15 counties in California (CDFA 2005), and currently is the most significant insect pest threatening the California grape industry (Purcell 1999, Purcell and Saunders 1999). It is distributed throughout the southern United States and into South America (Young 1958, Turner and Pollard 1959). It has recently invaded the islands of Hawaii and Tahiti (Hoddle 2004), and may spread to the grape-growing regions of northern Argentina and the southern tip of Brazil (Peterson et al. 2003).

The biology, particularly the reproductive morphology and physiology, of *H. coagulata* remains largely unknown. Hummel (2006a) described the functional morphology and associated musculature of the ovipositor of female *H. coagulata* at both light and scanning electron microscopic (SEM) levels. The SEM study revealed many potentially important sensillae associated with oviposition. Hix (2001) and Hummel (2006a) described the oviposition behavior of *H. coagulata*. *H. coagulata* has two generations per year in Florida (Alderz 1980) and southern California (Blua et al. 1999), and two to three generations per year in Texas (Sanderson 1905) and Georgia (Turner and Pollard 1959).

Life history studies have been completed on many species of Cicadellidae (Poos 1932, Severin and Klostermeyer 1950, Palmiter et al. 1960, Raine 1960, Tonks 1960, Stoner and Gustin 1967, Nielson 1968, Nielson and Toles 1968, Duan and Messing 2000), indicating that the incubation period of eggs ranges from 6 to 28 d. The length of the instars ranges from 1 to 8 d (first), 3–8 d (second), 2–9 d (third), 3–10 d (fourth), and 5–13 d (fifth). Adults of some species live up to 148 d (Poos 1932). According to Turner and Pollard (1959), *H. coagulata* nymphs molt four times to develop into winged adults in ≈10 wk in the first and third generations.

[1] Current address: USDA–ARS, Knipling Bushland Livestock Insects Research Laboratory, 2700 Fredericksburg Rd., Kerrville, TX 78028 (e-mail: Natalie.Hummel@ars.usda.gov).

[2] Department of Entomology, University of California, One Shields Ave., Davis, CA 95616.

0013-8746/06/0920–0932$04.00/0

A large number of plant disease vectors belong to the leafhopper family Cicadellidae. Little research has been conducted on the reproductive physiology of female Cicadellidae. The female reproductive organs of Cicadellidae have been described for *Amblydisca gigas* Fowler (Snodgrass 1933), *Bothrogonia ferruginea* F. (Hayashi and Kamimura 2002), *Bothrogonia japonica* Ishihara (Matsuzaki 1975), *Dalbulus maidis* (Delong & Wolcott), *Graminella nigrifons* (Forbes) (Tsai and Perrier 1996), and *Peregrinus maidis* (Ashmead) (Tsai and Perrier 1993). The glands involved in endocrine regulation of *Idiocerus atkinsoni* Lethierry were identified by Bhola and Srivastava (1979). In males, the ultrastructure of *D. maidis* sperm was described by Cruz-Landim and Kitajima (1972), and spermatophores have been described for *B. ferruginea* (Hayashi and Kamimura 2002) and Cicadidae (Kubo-Irie et al. 2003). Courtship behavior and copulatory time have been described in *Empoasca fabae* (Harris) (Carlson and Hibbs 1970), *H. coagulata* (Hix 2001), and *Sophinia rufofascia* (Kuoh & Kuoh) (Duan and Messing 2000).

A description of the ovariole type and the oogenesis process is fundamental to understanding the number of egg cycles, egg-laying pattern, and potential fecundity of female *H. coagulata* during different seasons. Furthermore, this information can be used to develop a method to determine the timing and number of generations per year for this insect. According to Detinova (1967), population reproductive patterns can be assessed accurately by determining the physiological age of the females, largely based on the ovarian development. Therefore, we have developed a method to rank ovarian development of field-collected specimens to model the insects' reproductive patterns.

In this study, we describe the anatomy of the reproductive organs of female *H. coagulata* and develop an ovarian ranking system to determine the reproductive patterns of *H. coagulata* in southern California citrus groves. There are three objectives of this study: 1) to describe the anatomy and histology of the reproductive organs of the female *H. coagulata*, and the histological events of oogenesis; 2) to describe the act of copulation with respect to the union of the genitalia and spermatophore transfer; and 3) to develop an ovarian ranking system, based on the morphological and histological examination of the developing oocytes, that can be used to describe the seasonal reproductive activity of female *H. coagulata.*

Materials and Methods

Dissections. Specimens were collected from October 2001 to February 2005 from citrus at Agricultural Operations, the University of California at Riverside, CA (UCR Ag Ops). For dissection purposes, individuals were collected using beat-net and beating stick or were hand-picked on cool mornings from citrus, injected with 70% ethanol into the abdomen by using a microsyringe, and stored in 70% ethanol until dissected. In total, 993 specimens were dissected under a stereoscope (model MZ12.5, Leica Microsystems Inc., Bannockburn, IL) fitted with a light source (model L2, Leica Microsystems Inc.) and a camera lucida attachment (model 10446193, Leica Microsystems Inc.). A second light source (model 3600, Diax, Tensor Corp., Brooklyn, NY) was used to illuminate the drawing surface, and a photographic adapter was fitted for digital imaging. Digital images were captured using a digital camera (Coolpix 995, Nikon Inc., Melville, NY) and contrast and focus adjusted using Adobe Photoshop (Adobe Systems, San Jose, CA).

The reproductive organs of *H. coagulata* were identified following descriptions of the female reproductive organs of *D. maidis*, *G. nigrifons* (Tsai and Perrier 1996), and *P. maidis* (Tsai and Perrier 1993). However, the structure named as the spermatheca by Tsai and Perrier (1996) is probably the bursa copulatrix. In their illustrations, there is an unlabeled rounded portion of the common oviduct anterior to the bursa copulatrix, which is probably the actual spermatheca. The true location of the spermatheca was identified following descriptions of the reproductive organs of *B. ferruginea* by Hayashi and Kamimura (2002) and based on sperm smear preparations of the spermatheca and bursa copulatrix of females collected while in copula.

Ovarian Ranking. An ovarian rank was assigned to each specimen based on measurement of a specific set of morphological characters of the reproductive organs. These were measured using an ocular micrometer fitted to a stereoscope (Leica MZ12.5, Leica Microsystems Inc.). Each of the characters was assigned as a variable. The variables measured include the following: variable 1, the number of visible oocytes per ovariole (oocov): a discrete variable; variable 2, the length of the largest oocyte (eggleng): a continuous variable; and variable 3, the presence or absence of a corpus luteum in each ovariole (corplut): an ordinal variable. The corpus luteum is formed when ovulating eggs shed the follicular epithelium, leaving a proteinaceous plug. Methylene blue stain (1% in 70% ethanol) was used to identify the corpora lutea in the ovarioles. The corpora lutea stain dark blue. Variable 4 was the diameter of the median accessory gland (glandiam) at the point where it enters the genital chamber: a continuous variable.

Once dissected, the four variables of the ovarioles were scored. Assignment of an ovarian rank to field-collected females is based on the combination of scores for each variable in each individual as described in Table 1. The ovarian ranks range from 0 to 7, with 0 being the youngest adult stage and 7 being the oldest adult stage.

Statistics. In Table 1, we present the mean and standard deviation for each variable in each rank, or for corpus luteum, the proportion of specimens in each rank with a corpus luteum. Because the data do not meet the assumptions of normality and the variables are of different types, analysis of variance (ANOVA) was not appropriate. Instead, a principal component analysis was performed to assess which variables would contribute to the components and

Table 1. Criteria used to assign ovarian ranks to field-collected specimens of *H. coagulata* (mean ± SD)

Rank	Vitellogenic status	No. oocytes[a]	Egg length[b]	Corpus luteum[c]	Gland[d]	n^e	Notes
0	Pre	0	N/A	0.0	0.12 ± 0.03	78	Small organs, bursa copulatrix clear
1	Pre	1.4 ± 0.5	0.27 ± 0.15	0.1	0.15 ± 0.05	59	One primary oocyte per ovariole
2	Pre	2.6 ± 0.8	0.78 ± 0.41	0.2	0.24 ± 0.07	23	Two oocytes per ovariole and no corpus luteum
3	Vitellogenic	3.0 ± 0.5	2.09 ± 0.43	0.8	0.28 ± 0.07	48	One mature ova per ovariole
4	Vitellogenic	3.1 ± 0.4	2.11 ± 0.28	0.8	0.30 ± 0.06	136	One mature and one developing oocyte per ovariole
5	Vitellogenic	2.2 ± 0.4	1.00 ± 0.66	1.0	0.25 ± 0.05	61	One mature or maturing oocyte per ovariole
6	Post	2.0 ± 0.2	0.36 ± 0.17	1.0	0.21 ± 0.05	218	One corpus luteum per ovariole
7	Post	1.1 ± 0.3	0.34 ± 0.18	0.9	0.20 ± 0.05	67	Two corpora lutea per ovariole

[a] Number of oocytes per ovariole.
[b] Length (millimeters) of the largest egg in each ovariole. N/A, not applicable.
[c] Proportion with a corpus luteum present.
[d] Diameter (millimeters) of the median accessory gland at the base.
[e] ntotal = 690.

how the data could be clustered independently of the ovarian rank assignment. We used princomp (SAS Institute 1998) to calculate the principal component scores to determine the distribution of the ovarian ranks according to the value of each of the four variables. S-Plus (Insightful Corporation 2005) was used to generate biplots of the first principal component versus the second principal component, and the first principal component versus the third principal component. Each variable was plotted as a vector.

Histology. To verify the ovarian ranking method, ovarioles were examined histologically. Female *H. coagulata* were initially preserved in 70% ethanol. They were later rehydrated through a graded dilution series of ethanol of 50%, and 30% for 15 min each, and fixed in Bouin's fixative (product no. 16045, Polysciences, Inc., Warrington, PA) for at least 7 d. Specimens were then washed in deionized water for 48 h with two changes of water; dehydrated through a graded series of ethanol at 30, 50, 70, 95, and 100% for 15 min each, and cleared in xylene for 1 min. Once cleared, each specimen was infiltrated with paraffin wax (Paraplast tissue embedding medium, Fisher, Inc., Pittsburgh, PA) in a small watch glass for 72 h under 20-psi vacuum at 65°C. Specimens were placed in paraffin block embedding trays (catalog no. 26250, Lab-Line Biomedical Products, Inc., Melrose Park, Ill.), and the wax was allowed to harden for at least 24 h. Paraffin blocks were sectioned into ribbons of 0.5-μm thickness. These ribbons were floated in a warm water bath and captured onto glass slides (catalog no. 12-544-3, Fisher) previously coated with albumin adhesive solution. The slides were dried on a warming plate for ≈6 h and placed in slide boxes and stored at 20°C (68°F) until stained. Slides were stained with Harris modified hematoxylin with acetic acid (SH26-500D, Fisher) by using a progressive staining procedure, coverslips were mounted with Permount mounting medium (catalog no. 17986-01, Electron Microscopy Science, Ft. Washington, PA), and slides were dried in a chemical fume hood for 24 h on a warming plate. Stained slides were examined under a phase-contrast compound microscope (Nikon Optiphot-2, Nikon Inc.). Digital images were taken and contrast and focus adjusted using Adobe Photoshop (Adobe Systems).

Regions of the ovarioles were named according to the descriptions of Bonhag (1955) and Bonhag and Wick (1953) for *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae) and Matsuzaki (1975) for *Bothrogonia japonica* (Hemiptera: Cicadellidae).

Copulation, Sperm Transfer, and Sperm Morphology. Mating pairs were hand-captured on citrus at UCR Ag Ops in March 2004, by gently tapping them from the plant into a 20-ml glass scintillation vial (FS74504-20, Fisher) containing a small citrus leaf. Some pairs separated when disturbed, but others remained in copula. Mating pairs were held for up to 30 min in a small cooler with blue ice before being killed by using an ultracold solution of 95% acetone and dry ice. This ultracold solution froze the pair in copula, allowing for microscopic examination of the union of the genitalia during copulation. Once the pairs were dead, they were transferred to 70% ethanol to prevent desiccation of internal organs. Specimens were dissected under a stereoscope (model MZ12.5, Leica Microsystems Inc.) fitted with a photographic adapter for digital imaging. Digital images were captured using a digital camera (Coolpix 995, Nikon Inc.) and contrast and focus adjusted using Adobe Photoshop (Adobe Systems).

Some mating pairs were kept alive, and females were later dissected in Ringer's solution. The bursa copulatrix was dissected and the spermatophore was removed. The spermatheca also was dissected and squashed for preparation of sperm smears. Some males also were dissected, and the testes were squashed for preparation of sperm smears. The sperm smear was prepared by placing the spermatophore, spermatheca, or testes in a droplet of Ringer's solution on a glass slide. A second glass slide was then pressed onto the spermatophore, spermatheca, or testes, and pressure was applied to smear the sample across the two slides. A droplet of 1% eosin in 70% ethanol was spread on each slide to stain the sperm (Peng et al. 1990). Slides were placed in a slide box and allowed to air dry for at least 7 d. Coverslips were mounted using Permount mounting medium (catalog no. 17986-01, Electron Microscopy Science). These slides were examined for the presence of sperm under a compound microscope (Nikon Optiphot, Nikon Inc.), and digital images were

Table 2. Four principal components (PC) derived from the ovarian rank data set

Variable	First PC	Second PC	Third PC	Fourth PC
Corpus luteum (corplut)	0.352	−0.901	−0.127	−0.220
No. oocytes per ovariole (oocov)	0.567	0.000	−0.254	0.781
Large egg length (eggleng)	0.521	0.411	−0.484	−0.571
Gland diam (glandiam)	0.523	0.122	0.828	−0.128

captured and contrast and focus were adjusted using Adobe Photoshop (Adobe Systems).

Results

Anatomy of Female Reproductive Organs. The reproductive organs of female *H. coagulata* (Fig. 6) consist of two ovaries, each of which contains eight to 10 telotrophic ovarioles. The pedicel of each ovariole joins basally into a calyx, which opens into a lateral oviduct. The paired lateral oviducts join at the common oviduct (Fig. 6). A small ball-shaped swelling of the common oviduct is the spermatheca. Posterior to the spermatheca is the enlarged genital duct. The bursa copulatrix is a complex pouch with four compartments (Fig. 24) that branches off from the enlarged genital duct (Figs. 6 and 20, 21). The vagina is posterior to the bursa copulatrix and opens into the genital chamber by way of the gonopore. A single median accessory gland (Fig. 8) and a pair of small bead-like glands that have the appearance of a string of pearls (Fig. 11) also open into the genital chamber. The final exit of the reproductive canal is the genital pore that opens into the genital chamber. The latter is an invagination anterior to the reduced sternite VIII (Hummel 2006a). The opening of the genital chamber serves both as the vulva (a copulation opening) and the ovipore (an egg exit opening) (Fig. 6).

Ovarian Rank. We developed a method to reliably assign ovarian rank to field-collected females. Females assigned an ovarian rank 0, 1, or 2 are previtellogenic (Figs. 5 and 6, 7). These specimens are primarily distinguished by the absence of corpora lutea in the ovarioles. Ovarian rank 0 females have no visible oocytes per ovariole, no corpus luteum, the germarium is round, and the accessory gland is small (mean diameter, 0.12 mm; $n = 78$) (Fig. 5). Ovarian rank 1 females have one small primary oocyte per ovariole, no corpus luteum, and the accessory gland diameter is small (mean, 0.15 mm; $n = 59$) (Fig. 6). Ovarian rank 2 females have two oocytes per ovariole, no corpus luteum, the largest egg in the ovariole may be about one-half the length of a mature oocyte (mean length, 0.78 mm; $n = 23$), and the accessory gland has a medium-sized diameter (mean, 0.24 mm; $n = 23$) (Fig. 7).

Females assigned an ovarian rank of 3, 4, or 5 are vitellogenic (Figs. 8 and 9, 10). Vitellogenic females are primarily distinguished by the presence of a "sausage-shaped" (Turner and Pollard 1959) mature egg in the common oviduct. The length of the largest egg is much greater in vitellogenic females than in previtellogenic or postvitellogenic females. Ovarian rank 3 females have two immature oocytes and one mature egg (mean length, 2.09 mm; $n = 48$) in each ovariole (Fig. 8). The accessory gland is large (mean diameter, 0.24 mm; $n = 48$), and a corpus luteum may be present or absent in each ovariole. Ovarian rank 4 females have one immature (primary) oocyte, one maturing oocyte (undergoing vitellogenesis), and one mature egg (mean length, 2.11 mm; $n = 136$) in each ovariole (Fig. 9). The accessory gland is the largest (mean, 0.30 mm; $n = 136$) in ovarian rank 4 specimens. Ovarian rank 5 females have one primary oocyte, one medium to large maturing egg (mean length, 1.0 mm; $n = 61$), and a corpus luteum in each ovariole (Fig. 10). The accessory gland is medium to large (mean diameter, 0.25 mm; $n = 61$).

Ovarian ranks 6 and 7 are postvitellogenic (Figs. 11 and 12) and are distinguished by having smaller eggs and a corpus luteum in each ovariole. Ovarian rank 6 females have two small oocytes (mean length of largest oocyte, 0.36 mm; $n = 218$) and a corpus luteum in each ovariole (Fig. 11). The accessory gland is medium sized (mean diameter, 0.21 mm; $n = 218$). Ovarian rank 7 females have one small, primary oocyte (mean length, 0.34 mm; $n = 67$) and a corpus luteum in each ovariole (Fig. 12). The accessory gland diameter is small to medium (mean, 0.20 mm; $n = 67$).

Principal Component Analysis. The principal component analysis revealed that there are four principal components in the data set (Table 2). The first principal component explains 66% of the variability in the data set, the second explains 20% of the variability (Fig. 1), the third explains 9% of the variability (Fig. 2), and the fourth explains 5% of the variability. The first principal component is an age-related reproductive status score. A larger score indicates a corpus luteum (corplut) is present, the number of oocytes per ovariole (oocov) is greater, the largest egg (eggleng) is longer, and the gland diameter (glandiam) is larger. The variables oocov and glandiam contribute the most to the scoring of the first principal component. Variable corplut does not play a large role in the scoring of the first principal component, because it changes very little along the x-axis. Along the x-axis, previtellogenic and postvitellogenic ranks are more abundant in the negative scores, and as scores increase in value there is an increasing abundance of vitellogenic ranks (Fig. 1).

The second principle component is an age score. There are three discrete divisions in the scores along the y-axis (Fig. 1). The distribution of the scores of the second principal component seems to be related to the presence or absence of a corplut. As the proportion of specimens with a corplut increases, the abundance of

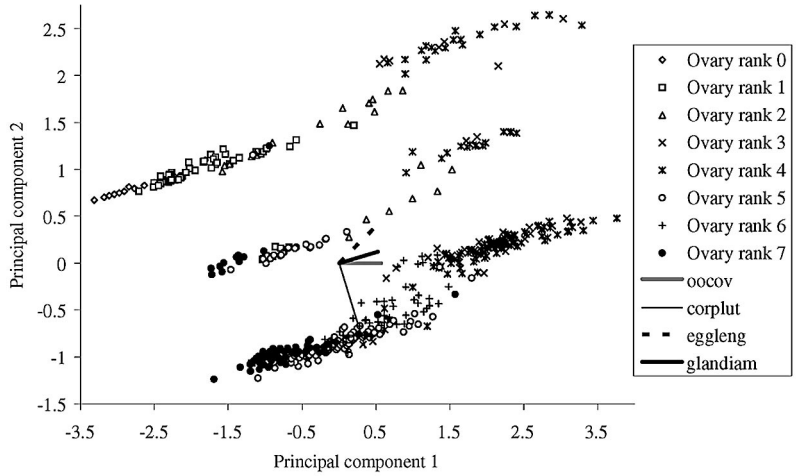

Fig. 1. Biplot of the distribution of the ovarian ranks according to the first principal component scores and the second principal component scores.

postvitellogenic ranks increases. Eggleng is also important in calculating the scores of the second principal component. As the value of eggleng increases, the abundance of vitellogenic females increases.

The third principal component is a current reproductive status score. Glandiam is the most important variable of this component (Fig. 2). The distribution of ranks is similar to the slope of the glandiam vector. Along the x-axis (first principal component), the distribution of the ranks starts with previtellogenic and increases to postvitellogenic females. The most positive scores are associated with vitellogenic ranks.

Descriptive Statistics. Variable 1, the number of oocytes per ovariole, was different when contrasting ovarian ranks 0, 1, 6, and 7 with ovarian ranks 2, 3, and 4 (Fig. 3). This variable is important only when considered in addition to the presence or absence of a corpus luteum. It is not possible to distinguish between previtellogenic and postvitellogenic females based solely on the number of oocytes per ovariole.

Variable 2, the length of the largest egg in each ovariole, was different when contrasting ovarian ranks 1, 2, 5, 6, and 7 with ovarian ranks 3 and 4 (Figs. 4 and 8, 9). The length of the largest oocyte is the most important characteristic to distinguish vitellogenic females from previtellogenic or postvitellogenic females, because large, mature eggs are only found in vitellogenic females.

Variable 3, the presence or absence of a corpus luteum in each ovariole, was different when contrasting ovarian ranks 0, 1, and 2 with ovarian ranks 3–7 (Fig. 3). A corpus luteum is always absent in previtellogenic females but is present in all postvitellogenic females and in some vitellogenic females. This variable is sometimes difficult to distinguish when using a stereoscope. Therefore, a low propor-

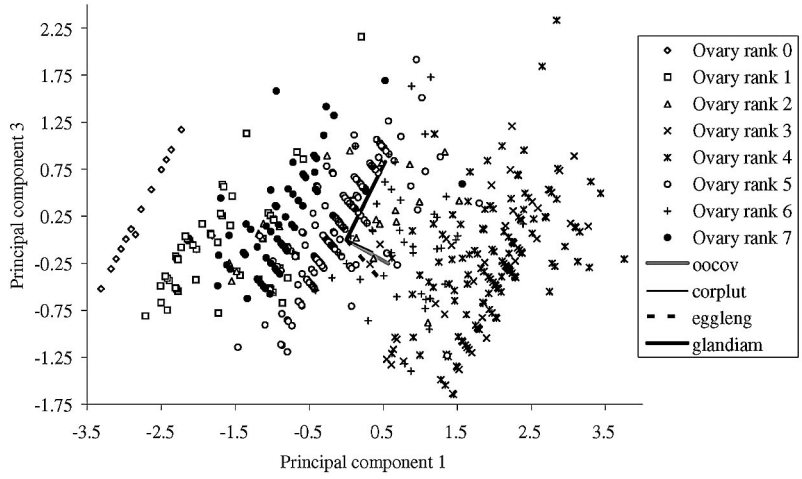

Fig. 2. Biplot of the distribution of the ovarian ranks according to the first principal component scores and the third principal component scores.

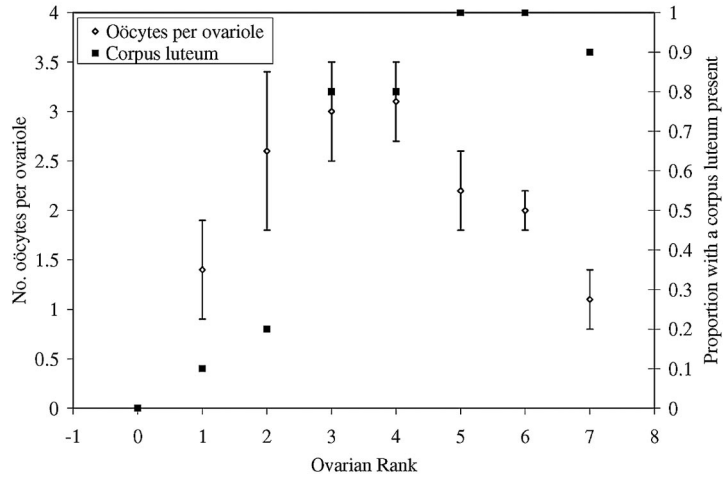

Fig. 3. The number of oocytes per ovariole (oocov) (mean ± SD) and proportion with a corpus luteum (corplut) present in relation to the ovarian rank of female *H. coagulata*, collected from citrus hosts in Riverside, CA (n = 690).

tion of females in ovarian ranks 1 and 2 have a corpus luteum present.

Variable 4, the diameter of the median accessory gland, increases as females mature and begin ovulation. After ovulation, the accessory gland diameter decreases (Table 1). As postvitellogenic females mature subsequent egg batches, the diameter of the median accessory gland increases once again (Fig. 4). The diameter of the median accessory gland is most useful when contrasting ovarian rank 0 females with vitellogenic and postvitellogenic females. The function of the median accessory gland is unknown and merits further study.

Histology. Histological examination reveals that *H. coagulata* have telotrophic ovarioles (Fig. 13–16). The ovarioles have four regions: the terminal filament (TF), the germarium (G), the vitellarium (V), and the pedicel (P). The most anterior region of the ovarioles is the terminal filament (Figs. 6 and 15). A transverse septum (TS) separates the terminal filament from the germarium region (Figs. 14 and 15). The germarium (Fig. 13) contains the trophocytes and primordial oocytes (PO) and has three zones of cell division (Fig. 14). In zone I (ZI) the trophic cells are small and most likely contain a single nucleus, with indistinguishable nuclear boundaries (Fig. 14). Zone II (ZII) is located posterior to zone I, and the cells are larger (Fig. 14). In zone II, the trophocytes are undergoing mitotic growth. Zone III (ZIII) forms the posterior two-thirds of the germarium and contains trophocyte nuclei arranged in aggregates around the central trophic core (TC) (Figs. 13 and 14). In zone III, the trophic cell nuclei are larger, and cell boundaries are lost, forming a large trophic core (Figs. 13 and 14). Nutritive cords (NC) arise from the posterior region of the trophic core and form connections with the primordial oogonia (POO) (Fig. 16).

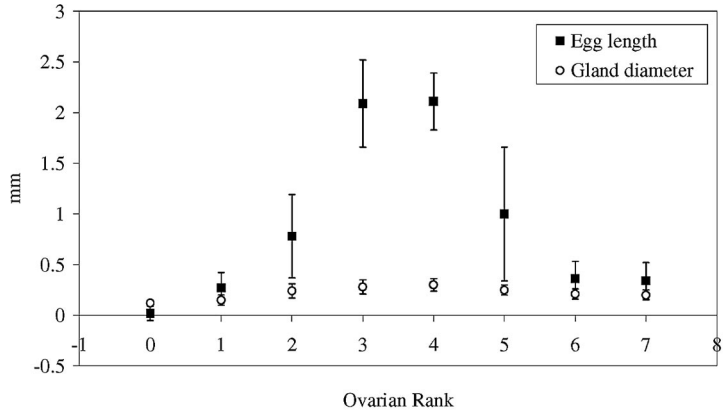

Fig. 4. Median accessory gland diameter (millimeters) (mean ± SD) and length (millimeters) (mean ± SD) of the largest egg in the ovarioles in relation to the ovarian rank of female *H. coagulata*, collected from citrus hosts at Riverside, CA (n = 690).

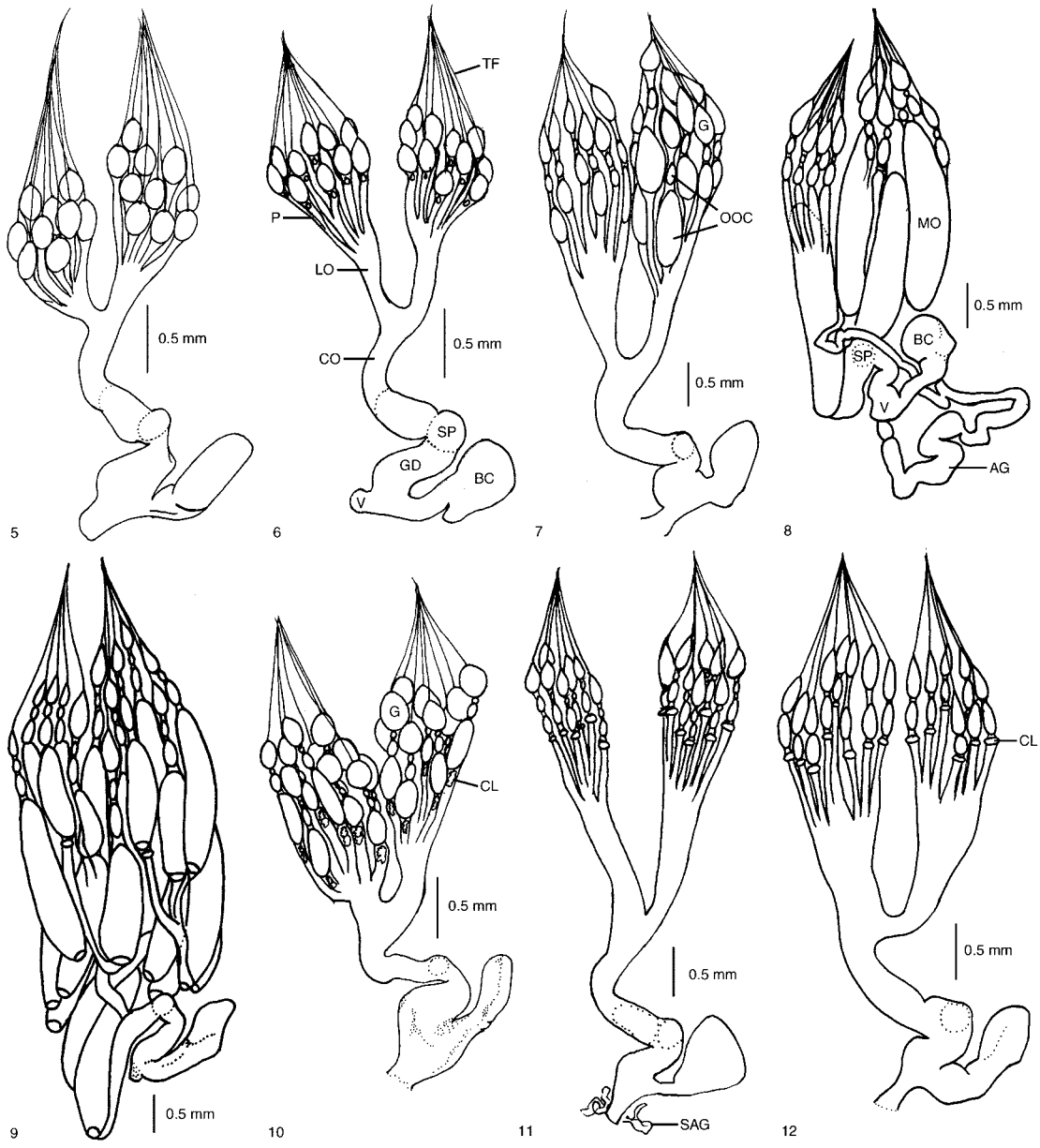

Fig. 5–12. Illustrations of the reproductive organs of female *Homalodisca coagulata* at different stages of reproductive activity. [5] The reproductive organs of a previtellogenic female *H. coagulata* assigned an ovarian rank zero, collected 2 November 2004. [6] The reproductive organs of a previtellogenic female *H. coagulata* assigned an ovarian rank 1, collected 21 October 2004. BC, bursa copulatrix; CO, common oviduct; GD, enlarged genital duct; LO, lateral oviduct; P, pedicel; SP, spermatheca; TF, terminal filament; and V, vagina. [7] The reproductive organs of a previtellogenic female *H. coagulata* assigned an ovarian rank 2, collected 17 June 2004. G, germarium; and OOC, oocyte. [8] The reproductive organs of a vitellogenic female *H. coagulata* assigned an ovarian rank 3, collected 4 August 2004. AG, median accessory gland; BC, bursa copulatrix; MO, mature oocytes; SP, spermatheca; and V, vagina. [9] The reproductive organs of a vitellogenic female *H. coagulata* assigned an ovarian rank 4, collected 5 March 2004. [10] The reproductive organs of a vitellogenic female *H. coagulata* assigned an ovarian rank 5, collected 17 February 2004. CL, corpus luteum; and G, germarium. [11] The reproductive organs of a postvitellogenic female *H. coagulata* assigned an ovarian rank 6, collected 2 November 2004. SAG, small accessory glands. [12] The reproductive organs of a postvitellogenic female *H. coagulata* assigned an ovarian rank 7, collected 22 July 2004. CL, corpus luteum.

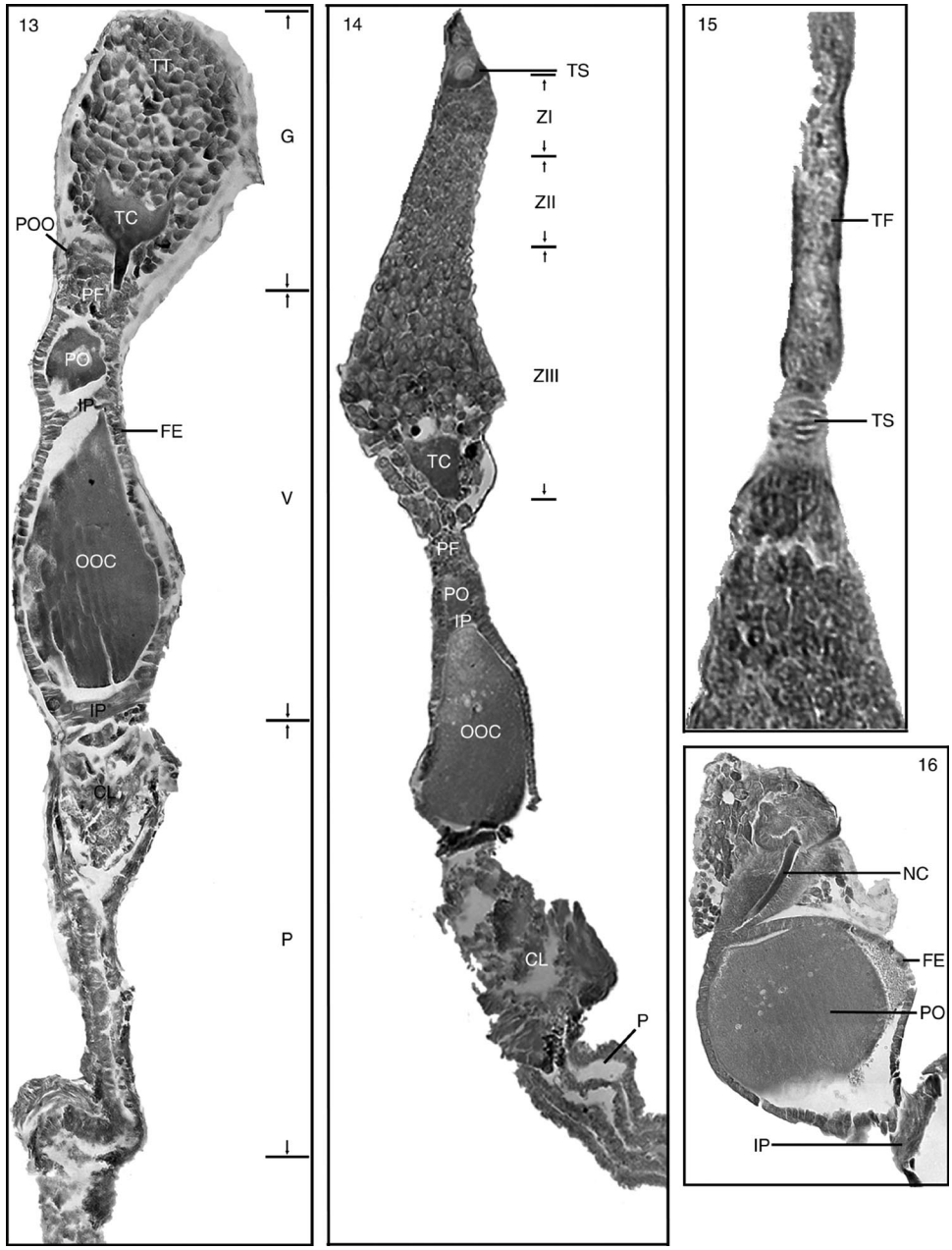

Fig. 13–16. Longitudinal sections of the reproductive organs of a female *H. coagulata* stained using the progressive hematoxylin procedure. [13] Longitudinal section of a telotrophic ovariole of a postvitellogenic female *H. coagulata*. CL, corpus luteum; FE, follicular epithelium; G, germarium; IP, interfollicular plug; OOC, oocyte in the fast-growing phase; P, pedicel; PF, prefollicular tissue; PO, primary oocyte; POO, primordial oogonia; TC, trophic core; TT, trophic tissue; V, vitellarium, YO, young oocytes. [14] A longitudinal section of a single telotrophic ovariole of a postvitellogenic *H. coagulata*. CL, corpus luteum; IP, interfollicular plug; OOC, oocyte in the fast-growing phase; P, pedicel; PF, prefollicular tissue; PO, primary oocyte; TC, trophic core; TS, transverse septum; ZI, zone I of germarium; ZII, zone II of germarium; ZIII, zone III of germarium. [15] A longitudinal section of the terminal filament (TF) of a telotrophic ovariole of a female *H. coagulata*; TS, transverse septum. [16] A longitudinal section of a primary oocyte (PO) of a female *H. coagulata* with an anterior nutritive cord (NC) and a posterior interfollicular plug (IP); FE, follicular epithelium.

The trophocytes and trophic core are followed by the prefollicular tissue and primordial oogonia (Figs. 13 and 14). As each young oocyte exits the germarium, it is enclosed in prefollicular cells (PF) and connected to the trophocytes by way of a nutritive cord (Fig. 16). The prefollicular tissue is devoid of

cellular boundaries and looks like small circles (Figs. 13 and 14).

The germarium is followed by the vitellarium that contains the developing oocytes, beginning with the primary oocytes (PO) (Figs. 13 and 14, 16). During vitellogenesis, the oocytes increase in size as yolk is deposited (Figs. 13 and 14). Attached to the anterior tip of each oocyte is a nutritive cord, which transports nutrients from the anterior nurse cells to each developing oocyte (Fig. 16).

Egg maturation is synchronized with all of the ovarioles simultaneously maturing one oocyte per ovariole (Fig. 9) in both ovaries. Occasionally, ovarioles simultaneously mature two oocytes per ovariole. Most often two oocytes are observed in each ovariole: the primary oocyte and a maturing oocyte. The primary oocyte is in the slow-growing phase and is followed by the maturing oocyte in the fast-growing phase (OOC) (Figs. 9 and 13, 14). The developing oocytes are lined with follicular epithelia (FE) (Figs. 13 and 16). Each developing oocyte is separated from the next by an interfollicular plug (IP), which probably contains trapped prefollicular cells (Figs. 13 and 14, 16) (Bonhag and Wick 1953). Posterior to the largest oocyte is the epithelial plug, which ruptures when the first egg is ovulated (Bonhag and Wick 1953). After ovulation, the follicular epithelium collapses forming the corpus luteum (CL) (Figs. 10 and 11, 12, 13, 14). Each ovariole opens into a pedicel (P), which, in turn, opens into the lateral oviduct (LO) (Figs. 6 and 13, 14).

Histological examination verified that females that have not yet ovulated eggs are in the previtellogenic phase, and their ovarioles contain small, primary oocytes. Females that are ready to ovulate are in the vitellogenic phase and possess a mature oocyte in each ovariole (Figs. 8 and 9). Females that have ovulated are in the postvitellogenic phase and there is a corpus luteum in each ovariole (Figs. 10 and 11, 12, 13, 14).

Copulation, Sperm Transfer, and Sperm Morphology. Before copulation, the male and female approach each other resting side by side with both heads facing the same direction. The female climbs onto the dorsum of the male and they change their orientation to tail to tail (Fig. 17). The female is still above the male when copulation is initiated, and both insects have their anal styli oriented perpendicular to the plant stem. The female strokes the male abdomen with her hindlegs along the long body axis from the base of the abdomen to the apex. Then, both slowly lower their abdomens toward the plant stem. The female moves her hindlegs in small circles lateral to the male abdomen and finally rests them on the stem. While in copula, the pair remains parallel to the stem and is responsive to observers. If the mating pair senses a threat, they hide by moving around the plant stem to the side opposite the intruder. During copulation the pair changes their location on the plant, moving first to another leaf, and finally moving to the apex of the plant stem in the sun. Mating was observed to last up to 106 min. After mating, the male and female separate and the female commences feeding while holding her hindlegs, abdomen, and anal stylus away from the plant stem.

The female external genitalia have been described by Hummel (2006a). Male genitalia are enclosed laterally by a pair of claspers (CS) and ventrally by two triangular genital plates (GP) (Fig. 19). The phallus (P) joins basally with the parameral processes (PP). At the distal apex of the phallus there are two horn-shaped harpagones (H) located dorsally and two serrated apical processes (AP) located ventrally (Fig. 19). During copulation, the female pygofer (PY) and the male genitalia are positioned perpendicular to the long axis of the body. Laterally, the male claspers are positioned posterior to the female sternite VII, which is enclosed ventrally by the male genital plates (Fig. 18). In this perpendicular orientation, the female pygofer bends dorsally causing the genital chamber to open. Once the female sternite VII (S-VII) is bent ventrally and the pygofer is bent dorsally, the male claspers grasp the female's eighth paratergites. The male-paired apical processes are then inserted anteriorly under the lateral corners of sternite VII. The paired harpagones are positioned posteriorly, under the anterior margin of the first valvifer and the remnants of sternite VIII (Fig. 18) (Hummel 2006a).

The spermatophore (SPO) is formed in the bursa copulatrix (BC) during copulation (Figs. 20 and 21). Females that were captured in copula were dissected in Ringer's solution to confirm the presence of a spermatophore in the bursa copulatrix. The spermatophore is typically pear-shaped with the narrow portion located at the posterior neck of the bursa copulatrix (Figs. 20 and 21). It is clear and gelatinous in appearance and resistant to tearing by using a micro-minuten probe. Occasionally, the spermatophore has the appearance of a pear with two horns. One horn is projected into the bursa copulatrix and the other horn into the enlarged genital duct (GD). The actual size of the spermatophore varies considerably among females. The variation we observed may be related to the duration of mating and whether mating was interrupted. After preservation in 70% ethanol, the appearance of the spermatophore changes from a large clear pear to a tan or white ball of tissue and if somewhat digested, it is found in the folds of the bursa copulatrix wall (Fig. 21). From March 2004 to February 2005, nearly all females collected were found to contain spermatophore remnants in the bursa copulatrix, indicating that these females had mated. This finding suggests that mating occurs very soon after eclosion into the adult stage.

The filamentous sperm of *H. coagulata* have the appearance of typical insect sperm, with a small head and a long flagellum (Figs. 22 and 23). The total length of a sperm is ≈200 μm, the head is ≈35 μm in length, and the tail is ≈165 μm in length.

Discussion

The female reproductive organs of *H. coagulata* are similar to those of other Cicadellidae, including *A. gigas* (Snodgrass 1933), *D. maidis*, *G. nigrifons* (Tsai

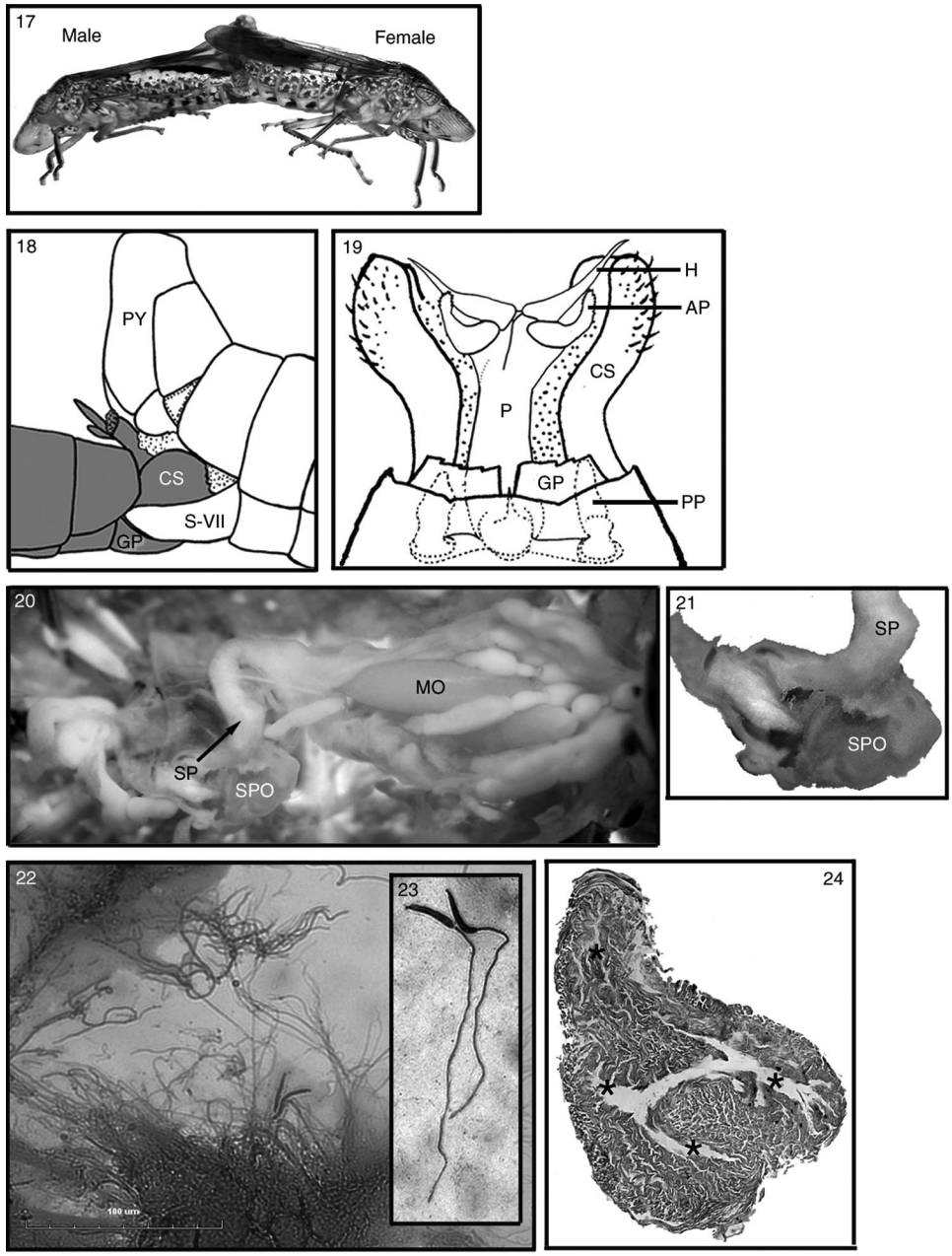

Fig. 17–24. [17] Lateral view of a *H. coagulata* pair in copula with the male on the left and the female on the right. [18] Illustration of the distal abdominal segments of an *H. coagulata* pair in copula showing the interlocking of external genitalia, male in gray and female in white; CS, male claspers; GP, male genital plates; PY, female pygofer; and S-VII, female sternite VII. [19] Illustration of a ventral view of the genitalia of a male *H. coagulata*; AP, apical processes; CS, claspers; GP, genital plates; H, harpagones; P, phallus; and PP, parameral processes. [20] The reproductive organs of a vitellogenic female *H. coagulata* that has mated, showing the appearance of a bursa copulatrix containing a spermatophore (SPO) and the ball-shaped spermatheca (SP); MO, mature oocyte. [21] The sperm storage organs of a female *H. coagulata*; SP, spermatheca, and SPO, spermatophore located in a dissected bursa copulatrix. [22] A sperm smear from the bursa copulatrix of a female *H. coagulata*. [23] Two *H. coagulata* sperm cells showing the size of the head with respect to the tail. [24] Cross-section of the bursa copulatrix of a female *H. coagulata* with four chambers (*) stained with hematoxylin progressively.

and Perrier 1996), *P. maidis* (Tsai and Perrier 1993), and *B. japonica* (Matsuzaki 1975). There are eight to 10 telotrophic ovarioles per ovary, similar to that of *Nephotettix cincticeps* Uhler and *Nephotettix virescens* (Distant) (Nasu 1965). Matsuzaki (1975) reported that *B. japonica* has 12 telotrophic ovarioles per ovary, with each ovariole containing an anterior terminal filament, a germarium region, a vitellarium region, and a pedicel. Oocytes within the vitellarium region were divided into five developmental stages by Matsuzaki (1975): stages 1 to 3 were previtellogenic; stage 4 was a vitellogenic stage, actively uptaking lipid and forming yolk proteins; and stage 5 was undergoing maturation as egg membranes were being formed. The present histological study reveals a similar oocyte maturation pattern in the ovarioles of *H. coagulata.*

We developed a method to assign ovarian ranks to field-collected specimens. The assignment of eight ovarian ranks is supported by histological evidence and the principal component analysis. Histology verified the number of oocytes per ovariole and the presence or absence of corpora lutea in the ovarioles. Corpora lutea are absent in ovarioles that were assigned to previtellogenic ranks but were present in ovarioles assigned to postvitellogenic ranks.

Histology verified that the ovarioles of *H. coagulata* are of the telotrophic type containing germarium and vitellarium regions. As in *H. coagulata*, nearly 80% of the germarium of *B. japonica* consists of trophic tissue anteriorly, with primordial oogonia located posteriorly (Matsuzaki 1975). Also, the boundaries of trophocyte nuclei of *H. coagulata* were difficult to distinguish as was seen in *B. japonica* (Matsuzaki 1975). The functions of the germarium zones of a telotrophic ovariole were detailed by Bonhag and Wick (1953) for *O. fasciatus. H. coagulata* ovarioles also seem to have three zones of development within the germarium region. In zone I, the undifferentiated cells are proliferating and undergoing mitosis, but posteriorly they are arrested mitotically. Zone II is a region of progressively larger binucleate trophocytes marked by loss of cell boundaries and irregular nuclear boundaries (Matsuzaki 1975) and contains the trophic core and degenerated trophocytes (Matsuzaki 1975). The arrangement of the nuclei around the central trophic core of *H. coagulata* is similar to that in *O. fasciatus* (Bonhag and Wick 1953) and *B. japonica* (Matsuzaki 1975).

In *H. coagulata*, the germarium region is followed by the vitellarium. Prefollicular tissue and the primordial oogonia are located between the germarium and vitellarium. The primordial oogonia develop into primary oocytes (Bonhag and Wick 1953). The young oocytes of *H. coagulata* form attachments to the nutritive cords as they exit the germarium region, as similarly described in *O. fasciatus* (Bonhag and Wick 1953) and *B. japonica* (Matsuzaki 1975). The nutritive cords may contain microtubules, which may be involved in transport of nutrients from the trophocytes to the developing oocytes as seen in *Rhodnius* bugs (Hemiptera: Reduviidae) (Huebner and Diehl-Jones 1993). In *B. japonica*, the early young oocytes are sandwiched between the nutritive core and the nutritive cords of further developed young oocytes (Matsuzaki 1975). Histological examination of *H. coagulata* ovarioles also verified the presence of a corpus luteum in ovarioles of postvitellogenic females that had ovulated eggs.

The present histological study reveals that oocyte development in the ovarioles of *H. coagulata* is synchronized interovarioly. Observations on the oviposition behavior of *H. coagulata* (Hummel 2006a) and the number of eggs per egg mass correlate with the observation of interovariole synchronization. Sanderson (1905) noted that *H. coagulata* deposited eggs side-by-side in batches of 10–15 forming a "blister" under the leaf surface. Turner and Pollard (1959) also indicated that the egg masses were deposited under the epidermis of the leaf in groups ranging from 11 to 20. Hix (2001) found that a mean of 12.5 eggs ± 0.8 SE were oviposited in the first egg batch. Our observation that each ovary contains ≈10 ovarioles, which would simultaneously mature one egg per ovariole, is consistent with the oviposition of 10–20 eggs per egg mass.

We found that mated female *H. coagulata* are present year-round in southern California. Turner and Pollard (1959) observed that mating of *H. coagulata* in Georgia occurred from March to September. Based on our data on the presence or absence of a spermatophore in the bursa copulatrix, *H. coagulata* may mate throughout the summer and into the fall in Riverside, CA. However, we did not observe mating behavior during this period. Mating also may be coincident with mass emergences of previtellogenic adults that are observed in June, October, and December (Hummel 2006b), when the majority of adults would mate ≈7 d posteclosion and store sperm for the rest of their life. This may explain why mating is not observed year-round, but mated females are collected year-round. Powers (1973) found that *Homalodisca lacerta* (Fowler) adults in southern California were active and feeding throughout the year. However, copulatory behavior ceased during November and December. During this period, the air temperature would permit copulatory behavior, but it is possible that the physiology of host plants had changed and specific nutrients were not present to support ideal nymphal development, and consequently copulation did not occur (Powers 1973). Powers (1973) suggested that *H. lacerta* undergo atherno-oligopause (Mansingh 1971).

H. coagulata copulatory behavior is similar to that described for other species of Cicadellidae. Hix (2001) reported that female *H. coagulata* mated once 4–14 d posteclosion, whereas males mated multiple times. Powers (1973) found that female *H. lacerta* began development of eggs 3 d posteclosion and started mating 6 d posteclosion. This pattern is similar to that observed in other leafhoppers that mate ≈7 d posteclosion (Raine 1960, Tonks 1960, Stoner and Gustin 1967, Nielson 1968, Nielson and Toles 1968). Hix (2001) found that *H. coagulata* courted for 6.2 ± 0.3 (mean ± SE) min and that copulation lasted 165 ± 5.0 min. We observed a pair in copula on a young Mandarin tree for ≈2 h. Copulation lasting >1 h also was

observed by Duan and Messing (2000) in *S. rufofascia.* Copulatory behavior of *H. coagulata* is similar to that described in a number of deltocephaline cicadellids, including *Acinopterus angulatus* Lawson (Deltocephalinae) (Nielson and Toles 1968), *Empoasca devastans* Distant (Kumar and Saxena 1978), and *Sophinia rufofascia* (Kuoh & Kuoh) (Duan and Messing 2000).

The long copulatory time observed in *H. coagulata* may permit partial digestion of the spermatophore by enzymes in the bursa copulatrix, allowing sperm to exit the bursa copulatrix and travel up the enlarged genital duct into the spermatheca before copulation ceases as seen in *B. ferruginea* (Hayashi and Kamimura 2002). This lengthened copulatory period has the potential to increase the reproductive success of the male (Parker and Simmons 1989). Insemination by a spermatophore also may provide the female with additional nutrients for egg maturation (Boggs 1990, Wheeler 1996).

There has been some confusion in the literature about the location of the spermatheca and the bursa copulatrix in Cicadellidae. This confusion may explain why spermatophore insemination has only been described in *B. ferruginea* (Hayashi and Kamimura 2002). Pear-shaped spermatophores, similar to those described in *H. coagulata*, have been described in *B. ferruginia* (Hayashi and Kamimura 2002) and Cicadidae (Kubo-Irie et al. 2003). As observed in the current study, remnants of spermatophores are often found in the bursa copulatrix of mated female *H. coagulata* that have been preserved in 70% ethanol. Further research is needed to clarify the role of the gelatinous material of the spermatophore. The morphology of the sperm of *H. coagulata* is similar to that of *D. maidis* (Cruz-Landim and Kitajima 1972) with the ratio of the length of the head to the length of the flagellum being ≈1:5. Transmission electron microscopy examination is needed to clarify the ultrastructure of *H. coagulata* sperm.

The ovarian rank method developed in this article was used to model the reproductive activity of *H. coagulata* populations in Riverside, CA, citrus groves based on dissections of field-collected female specimens. Clarification of the timing of reproductive events, including peaks in egg production activity and mating activity, would enhance the timing of chemical treatments, particularly ovicides, and the release of egg parasitoids.

Acknowledgments

We thank L. Kimsey and two anonymous reviewers for critical review of the manuscript, M. Watnick for assistance with statistical analysis, G. Ballmer for providing insect samples, M. Fields for assistance with dissections and digital images, A. Thacher for assistance with fieldwork, and C. Hummel and T. Martin for moral support. Funding was provided by the University of California Pierce's Disease Research Grant Program and the California Department of Food and Agriculture Pierce's Disease Research Program. This research was completed in partial fulfillment of the Ph.D. requirements of N.A.H. in the Department of Entomology, UC Davis.

References Cited

Alderz, W. C., and D. L. Hopkins. 1979. Natural infectivity of two sharpshooter vectors of Pierce's disease of grape in Florida. J. Econ. Entomol. 72: 916–919.

Alderz, W. C. 1980. Ecological observations on two leafhoppers that transmit the Pierce's disease bacterium. Proc. Fla. State Hortic. Soc. 93: 115–120.

Almeida, R. P., and A. H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera, Cicadellidae). J. Econ. Entomol. 96: 264–271.

Bhola, R. K., and K. P. Srivastava. 1979. Studies on the neuroendocrine system of the mangohopper, *Idiocerus atkinsoni* Leth. (Homoptera: Jassidae). Acta Biol. Acad. Sci. Hung. 30: 363–372.

Blua, M. J., P. A. Phillips, and R. A. Redak. 1999. A new sharpshooter threatens both crops and ornamentals. Calif. Agric. 53: 22–25.

Boggs, C. L. 1990. A general model of the role of male-donated nutrients in female insects' reproduction. Am. Nat. 136: 598–617.

Bonhag, P. F., and J. R. Wick. 1953. The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). J. Morphol. 93: 177–283.

Bonhag, P. F. 1955. Histochemical studies of the ovarian nurse tissue and oocytes of the milkweed bug, *Oncopeltus fasciatus* (Dallas) I. Cytology, nucleic acids, and carbohydrates. J. Morphol. 96: 381–439.

[CDFA] California Department of Food and Agriculture. 2005. Distribution of GWSS in California. (http://www.cdfa.ca.gov/phpps/pdcp/gwmap.htm).

Carlson, O. V., and E. T. Hibbs. 1970. Oviposition by *Empoasca fabae* (Homoptera: Cicadellidae). Ann. Entomol. Soc. Am. 63: 516–519.

Costa, H. S., M. S. Blua, J. A. Bethke, and R. A. Redak. 2000. Transmission of *Xylella fastidosa* to oleander by the glassy-winged sharpshooter, *Homalodisca coagulata.* HortScience 35: 1265–1267.

Cruz-Landim, C., and E. W. Kitajima. 1972. The ultrastructure of male spermatozoa of corn leafhopper *Dalbulus maidis* Del. and W. (Homoptera: Cicadellidae). J. Submicrosc. Cytol. 4: 75–82.

Davis, M. J., S. V. Thomson, and A. H. Purcell. 1980. Etiological role of a xylem-limited bacterium causing Pierce's disease in almond leaf scorch. Phytopathology 70: 472–475.

Detinova, T. S. 1967. Age structure of insect populations of medical importance. Annu. Rev. Entomol. 12: 427–450.

Duan, J. J., and R. H. Messing. 2000. Mating, oviposition, and development of *Sophonia rufofascia* (Homoptera: Cicadellidae) in Hawaii. Ann. Entomol. Soc. Am. 93: 554–558.

Hayashi, F., and Y. Kamimura. 2002. The potential for incorporation of male derived proteins into developing eggs in the leafhopper *Bothrogonia ferruginea.* J. Insect Physiol. 48: 153–159.

Hewitt, W. B., B. R. Houston, N. W. Frazier, and J. H. Freitag. 1946. Leafhopper transmission of the virus causing Pierce's disease of grape and dwarf of alfalfa. Phytopathology 69: 393–395.

Hix, R. L. 2001. Egg-laying and brochosome production observed in glassy-winged sharpshooter. Calif. Agric. 55: 19–22.

Hoddle, M. S. 2004. The potential adventive geographic range of glassy-winged sharpshooter, *Homalodisca coagulata,* and the grape pathogen, *Xylella fastidiosa:* implica-

tions for California and other grape growing regions of the world. Crop Prot. 23: 691–699.

Huebner, E., and W. Diehl-Jones. 1993. Nurse cell-oocyte interaction in the telotrophic ovary. Int. J. Insect Morphol. Embryol. 22: 369–387.

Hummel, N. A., F. G. Zalom, and C.Y.S. Peng. 2006a. Structure of female genitalia of glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae). Arthropod Structure Dev. 35(2): 111–125.

Hummel, N. A., F. G. Zalom, N. C. Toscano, P. Burman, and C.Y.S. Peng. 2006b. Seasonal patterns of female *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) reproductive physiology in Riverside, California. Environ. Entomol. 35: 901–906.

Insightful Corporation. 2005. S-Plus, release 7.0 edition. Insightful Corporation, Seattle, WA.

Kubo-Irie, M., T. Nakazawa, and H. Mohri. 2003. Ultrastructure and function of long and short sperm in Cicadidae (Hemiptera). J. Insect Physiol. 49: 983–991.

Kumar, H., and K. N. Saxena. 1978. Mating behavior of the cotton leafhopper, *Empoasca devestans*, in relation to its age, ovarian development, diurnal cycle and CO_2 treatment. Ann. Entomol. Soc. Am. 71: 108–110.

Mansingh, A. 1971. Physiological classification of dormancies in insects. Can. Entomol. 103: 983–1009.

Matsuzaki, M. 1975. Ultrastructural changes in developing oocytes, nurse cells, and follicular cells during oogenesis in the telotrophic ovarioles of *Bothrogonia japonica* Ishihara (Homoptera, Tettigellidae). Kontyu 43: 75–90.

Nasu, S. 1965. Electron microscopic studies on transovarial passage of rice dwarf virus. Jpn. J. Appl. Entomol. Zool. 9: 225–237.

Nielson, M. W. 1968. Biology of the geminate leafhopper, *Colladonus geminatus*, in Oregon. Ann. Entomol. Soc. Am. 61: 598–610.

Nielson, M. W., and S. I. Toles. 1968. Observations on the biology of *Acinopterus angulatus* and *Aceratagallia curvata* in Arizona (Homoptera: Cicadellidae). Ann. Entomol. Soc. Am. 61: 54–56.

Palmiter, D. H., W. J. Coxeter, and J. A. Adams. 1960. Seasonal history and rearing of *Scaphytopius acutus* (Say) (Homoptera: Cicadellidae). Ann. Entomol. Soc. Am. 53: 843–846.

Parker, G. A., and L. W. Simmons. 1989. Nuptial feeding in insects: theoretical models of male and female interests. Ethology 82: 3–26.

Peng, C.Y.S., S. J. Locke, M. E. Nasr, T. P. Liu, and M. A. Montague. 1990. Differential staining for live and dead sperm of honey bees. Physiol. Entomol. 15: 211–217.

Peterson, A. T., R. Scachetti-Pereira, and D. A. Kluza. 2003. Assessment of the invasive potential of *Homalodisca coagulata* in western North America and South America. Biota Neotrop. 3: 1–7.

Poos, F. W. 1932. Biology of the potato leafhopper, *Empoasca fabae* (Harris), and some closely related species of *Empoasca.* J. Econ. Entomol. 25: 639–646.

Powers, N. R. 1973. The biology and host plant relations of *Homalodisca lacerta* (Fowler) in southern California. M.S. thesis, University of California, San Diego, CA.

Purcell, A. H. 1999. Prepared remarks for hearing of the California Assembly Agricultural Committee: Statement of Alexander H. Purcell. (http://www.CNR.Berkeley.EDU/xylella/ap.htm).

Purcell, A. H., and S. R. Saunders. 1999. Glassy-winged sharpshooters expected to increase plant disease. Calif. Agric. 53: 26–27.

Raine, J. 1960. Life history and behavior or the bramble leafhopper, *Ribautiana dtenerrima* (H.-S.) (Homoptera: Cicadellidae). Can. Entomol. 93: 10–20.

Sanderson, E. D. 1905. Miscellaneous cotton insects in Texas. U.S. Dep. Agric. Farmers' Bull. 223: 18–21.

SAS Institute. 1998. SAS/STAT user's guide, release 6.03 edition. SAS Institute, Cary, NC.

Severin, H.H.P., and E. C. Klostermeyer. 1950. *Colladonus geminatus* and *C. montanus:* life histories on virus infected and on healthy plants. Hilgardia 19:553–560.

Snodgrass, R. E. 1933. Morphology of the insect abdomen. Pt. II. The genital ducts and the ovipositor. Smithsonian Misc. Coll. 89: 86–91.

Sorenson, J. T., and R. J. Gill. 1996. A range extension of *Homalodisca coagulata* (Say) (Hemiptera: Clypeorrhyncha: Cicadellidae) to southern California. Pan-Pac. Entomol. 72: 160–161.

Stoner, W. N., and R. D. Gustin. 1967. Biology of *Graminella nigrifons* (Homoptera: Cicadellidae), a vector of corn (maize) stunt virus. Ann. Entomol. Soc. Am. 60: 496–505.

Tonks, N. V. 1960. Life history and behavior of the leafhopper *Macropsis fuscula* (Zett.) (Homoptera: Cicadellidae) in British Columbia. Can. Entomol. 93: 707–713.

Tsai, J. H., and J. L. Perrier. 1993. Morphology of the digestive and reproductive systems of *Peregrinus maidis* (Homoptera: Delphacidae). Fla. Entomol. 76: 423–436.

Tsai, J. H., and J. L. Perrier. 1996. Morphology of the digestive and reproductive systems of *Dalbulus maidis* and *Graminella nigrifons* (Homoptera: Cicadellidae). Fla. Entomol. 79: 563–578.

Turner, W. F. 1949. Insect vectors of phony peach disease. Science (Wash., DC) 109: 87–88.

Turner, W. F., and H. N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. U.S. Dep. Agric. Tech. Bull. No. 1188.

Wells, J. M, B. C. Raju, H.-Y. Hung, W. G. Weisburg, L. Mandelco-Paul, and D. J. Brenner. 1987. *Xylella fastidiosa* gen. nov., sp. nov: gram-negative, xylem limited, fastidious plant bacteria related to *Xanthomonas* spp. Int. J. Syst. Bacteriol. 37:136–143.

Wheeler, D. 1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41: 407–431.

Young, D. A. 1958. A synopsis of the species of *Homalodisca* in the United States (Homoptera, Cicadellidae). Bull. Brooklyn Entomol. Soc. 23: 7–13.

Received 4 November 2005; accepted 10 March 2006.